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A non-consensus branch point plays an important role in determining the stability of the 2-kb LAT intron during acute and latent infections of herpes simplex virus type-1

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Abstract

Herpes simplex virus type 1 (HSV-1) establishes lifelong latent infection in sensory neurons of the peripheral nervous system. During HSV latency, the latency-associated transcripts (LATs) are the only viral transcripts abundantly expressed. The most abundant form of LATs is a 2-kb stable intron spliced from a primary transcript (mLAT). It has been previously reported that a non-consensus branch point influences the stability of the intron (in vitro) in cells transfected with plasmid constructs (J. Virol. 71 (1997) 5849; J. Virol. 71 (1997) 4199). However, it is unknown whether this branch point is important in determining LAT stability in vivo (in the context of virus). To study the role of this stable intron in HSV-1 infection, we have constructed a mutant virus KOS-CONS in which the branch point has been mutated to consensus branch point nucleotides. The accumulation of the 2-kb intron in KOS-CONS-infected cells was greatly reduced. The LAT intron was not detectable in KOS-CONS-infected mouse trigeminal ganglia (TG) during acute and latent phase infection by Northern blot analysis. Replication of the KOS-CONS and the wild-type KOS viruses on Vero cells was determined to be similar, as was the level of HSV-1 DNA in mouse trigeminal ganglia during acute and latent phase infection. Using the mouse TG explant model, the reactivation pattern of both viruses was shown to be similar. Our data suggest that the unique branch point plays a significant role in determining the stability of LAT intron in vivo, but that the stability of the intron does not appear to affect HSV-1 replication, the establishment of latency, or viral reactivation.

Keywords: Non-consensus branch point; LAT; Herpes simplex virus type-1

Introduction

Herpes simplex virus type-1 (HSV-1) is a human pathogen that causes a lifelong latent infection in the peripheral nervous system. During latent infection of neurons, the HSV-1 latency-associated transcript (LAT) is produced at readily detectable levels. LATs are present as several non-polyadenylated collinear RNAs, including a major 2-kb LAT and a 1.5-kb splice variant (Spivack et al., 1991; Wagner and Bloom, 1997). The 2-kb LAT is believed to be a stable intron spliced from a low-abundance 8.3-kb primary transcript (mLAT) (Farrell et al., 1994). This intron is expressed during acute infection with late gene kinetics (Spivack and Fraser, 1988; Wagner et al., 1988). It lacks most of the structural characteristics of mRNA such as a cap or polyA tail (Dobson et al., 1989; Farrell et al., 1991; Mitchell et al., 1990). In contrast to other cellular introns that are rapidly degraded following excision from primary transcripts, the half-life of the 2-kb LAT intron has been measured to be 24 h in transiently transfected cells (Thomas et al., 2002).

The role of the LAT gene in the life cycle of HSV-1 is not clear. Studies on LAT function have been mainly focused on the 2-kb LAT intron because of its high abundance during latency and the presence of several potential open reading frames within the 2-kb LAT intron (Spivack et al., 1991; Wechsler et al., 1989). It has been suggested that the LAT plays a crucial role in viral reactivation. LAT mutant viruses show reduced ability to reactivate in the mouse model

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